Meeting

Lacto-N-Fucopentaose-1 (LNFP-1) and 2'-Fucosyllactose (2'-FL) Discussion Paper

Committee Paper for Discussion - ACNFP/153/02

Advisory Committee For Novel Foods and Processes

Application for Authorisation as a Novel Food for a mixture of Lacto-N-Fucopentaose-1 (LNFP-1) and 2'-Fucosyllactose (2'-FL).

Application number RP549

Issue

1. An application has been received under the novel food authorisation process (regulation 2015/2283 as repatriated) for a mixture of lacto-N-fucopentaose-1 (LNFP-1) and 2'-fucosyllactose (2'-FL), a source of human identical milk oligosaccharides.

2. The Committee is asked to advise on whether the available data provides an adequate basis for a risk assessment, and whether the novel food is safe and not nutritionally disadvantageous under the proposed use and use levels.

Background

3. On the 7th March 2021, the FSA received the submission for LNFP-1 and 2’-FL, a source of human identical milk oligosaccharides from Glycom A/S.

4. The novel food ingredient is made by the fermentation with the production micro-organism, followed by a series of purification, isolation and concentration steps to generate the purified novel food ingredient. The applicant intends to use

the novel food ingredient in conventional foods and beverages, infant formulas, follow-on formula, foods for infants and young children (including processed cereal-based food, baby foods, and milk-based drinks), foods for specific groups (including FSMPs and total diet replacement for weight control), and food supplements.

5. The application dossier is attached as Annex A and annex to the dossier is attached as Annex B. All contain confidential information. Also provided are the request for information made at the suitability check stage Annex C and the applicants response Annex D.

This application

Identification

6. The novel food ingredient corresponds to a mixture of oligosaccharides containing lacto-*N*-fucopentaose-1 (LNFP-1) and 2'-fucosyllactose (2'-FL). These oligosaccharides are identical in structure to the same molecules that are present in human milk – see Annex B [Annex I – confidential]. LNFP-I and 2'-FL are two of the most abundant individual oligosaccharides of the complex natural oligosaccharide fraction of human milk.

7. The novel food ingredient is produced by fermentation with the production micro-organism – see Annex B [Annexes II and III – confidential] – followed by a series of purification, isolation, and concentration steps. The final composition is verified by HPLC.

Production Process

8. The applicant states that the manufacturing process is largely comparable to the production processes of previously authorised human identical milk oligosaccharides (*i.e.*, 2'-FL, LNnT, 2'-FL/DFL, LNT, 3'-SL and 6'-SL) – Annex C: p15 dossier. An overview is shown in Table 2.b.2-1 (Annex A: p24 dossier).

Table 1. Overview of the Manufacturing Process for LNFP-I/2'-FL

Stage 1	**Upstream processing (USP)**

	Step 1	Media Preparation
	Step 2	Propagation
	Step 3	Seed Fermentation
	Step 4	Fermentation Phases
	Step 5	Removal of Microorganism
Stage 2		**Downstream processing (DSP)**
	Step 6	Purification/Concentration 1
	Step 7	Ion Removal
	Step 8	Decolourisation
	Step 9	Purification/Concentration 2
	Step 10	Drying
	Step 11	Sampling and Packaging
	Step 12	Quality Control & Batch Release

2'-FL = 2'-fucosyllactose; LNFP-I = lacto-N-fucopentaose I

9. Stage 1 of the process involves the conversion of D-lactose and D-glucose to LNFP-I/2'-FL by the adapted cellular metabolism of the production microorganism, which uses glucose as an exclusive energy and carbon source and lactose as a substrate for the biosynthesis. At the end of the fermentation, the production micro-organism is removed by filtration (Annex A: p18 – 19 dossier).

10. Stage 2 of the process involves a series of purification and isolation steps to generate the final high-purity ingredient (Annex A: p19 – 20 dossier).

11. The LNFP-I/2'-FL ingredient is manufactured in compliance with current Good Manufacturing Practice (cGMP) and the principles of Hazard Analysis Critical Control Point (HACCP) – see Annex A: p20 – 21 dossier and Annex B (quality control).

12. The production micro-organism is described in detail (Annex C: p16 – 17 dossier and Annex B [Annex II and III – confidential]).

13. The raw material and processing aids are listed in Table 2.b.1.2-1 (Annex C: p17 – 18 dossier). Specifications are found in Annex B [Annex IV – confidential]).

14. The applicant states that the quality control steps ensure that manufacturing by-products, impurities and contaminants are monitored. Analysis for the presence of biogenic amines, amino acids and their metabolites did not detect significant levels of these contaminants in the final product (Annex A: p21 dossier).

15. The applicant reports the absence of the production microorganisms in the bulk ingredient is demonstrated by testing of final batches for bacteria from the *Enterobacteriaceae* family according to internationally recognised method ISO 21528-2. Results reported in Table 2.b.1.3-1 (Annex A: p28 dossier). The updated CoA’s are found in Annex B [updated Annex V – confidential].

16. The applicant states that the absence of the production organism in the finished ingredient is also supported by analyses for residual DNA in final production batches. As demonstrated in Table 2.b.1.6-1, the absence of residual DNA from the production organism is confirmed by three different quantitative polymerase chain reaction (qPCR) methods (Annex A: p22 dossier).

Table 2. Levels of Residual DNA in Five Batches of LNFP-I/2'-FL Produced by Fermentation

Residual DNA by qPCR	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
futC assay	< LoQa	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ

IgtA assay	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ
galTK assay	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ
23S assay	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ	< LoQ

2'-FL = 2'-fucosyllactose; LNFP-I = lacto-*N*-fucopentaose I; NT = not tested (because not used in the production of this batch).

LOQa = 4 µg/kg (parts per billion)

17. The applicant reports the results from the analysis of anions and trace elements which indicate they are not present at any relevant degree. Heavy metals were analysed and found not to be a concern – see Table 3 below (Annex A: Table 2.b.1.6-2, p22 – 23 dossier). The updated CoA's are found in Annex D [updated Annex V – confidential].

Table 3. Levels of Anions, Trace Elements and Heavy Metals in Six Batches of LNFP-I/2'-FL Produced by Microbial Fermentation

Batch Number	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**	**Batch 6**
Phosphate (% w/w)	< 0.0010	< 0.0005	0.0004	< 0.0001	< 0.006	< 0.000584
Sulphate (% w/w)	0.11	0.0049	0.003	< 0.0044	0.0197	0.00619
Chloride (% w/w)	0.10	0.0435	0.0057	0.0044	0.0206	0.012757

Ammonium (% w/w)	< 0.0050	< 0.001	< 0.001	< 0.001	< 0.005	< 0.001
Potassium (% w/w)	0.0020	< 0.001	0.002	0.001	0.015	< 0.001
Sodium (% w/w)	0.1010	0.034	0.007	0.003	0.023	0.023
Calcium (% w/w)	0.0070	< 0.001	< 0.001	< 0.001	0.004	< 0.000
Lead (mg/kg)	< 0.01	< 0.01	0.01	< 0.01	0.01	< 0.01
Iron (mg/kg)	1	< 0.5	< 1	< 0.1	< 10	< 1
Copper (mg/kg)	< 0.1	0.2	0.3	0.1	< 0.1	< 0.2
Manganese (mg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	< 1.0	< 0.1
Zinc (mg/kg)	< 0.1	0.2	0.3	0.1	< 0.5	< 0.2
Selenium (mg/kg)	NT	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Molybdenum (mg/kg)	NT	< 0.1	< 0.1	< 0.1	< 0.2	< 0.1

Nickel (mg/kg)	NT	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Arsenic (total) (mg/kg)	< 0.1	< 0.1	< 0.1	< 0.1	0.170	< 0.100
Cadmium (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.001	< 0.010
Mercury (mg/kg)	< 0.01	< 0.01	< 0.01	< 0.01	< 0.005	< 0.010

2'-FL = 2'-fucosyllactose; LNFP-I = lacto-*N*-fucopentaose I; NT = not tested (because not used in the production of this batch).

18. The applicant reports the results from the analysis of microbial endotoxins and proteins which indicate that the levels detected were not a safety concern. The permitted level of endotoxins are specified by the applicant at ≤10 endotoxin units (EU)/mg (Annex C: p23 dossier). See Table 4. (Annex A: Table 2.b.1.6-3, p23 dossier). The updated CoA's are found in Annex D [updated Annex V – confidential].

Table 4. Batch Results for Microbial Endotoxins and Residual Proteins in LNFP-I/2'-FL

Batch Number	**Batch 1**	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**	**Batch 6**
Residual endotoxins (EU/mg)	0.1398	0.0023	0.0357	0.0107	< 0.0025	0.0012
Residual protein by Bradford assay (w/w %)	< 0.0017	< 0.0017	0.0091	< 0.0017	< 0.0017	< 0.0017

2'-FL = 2'-fucosyllactose; EU = endotoxin units; LNFP-I = lacto-N-fucopentaose I.

Composition and Specification

19. The applicant reports the results from the analysis of microbial endotoxins and proteins which indicate that the levels detected were not a safety concern. The permitted level of endotoxins are specified by the applicant at ≤10 endotoxin units (EU)/mg (Annex C: p23 dossier). See Table 2.b.1.6-3 (Annex A: p23 dossier). The updated CoA's are found in Annex D [updated Annex V – confidential].

Table 5. Batch Results for Carbohydrates in LNFP-I/2'-FL

Parameter	Specification	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Average
Assay (water-free) – LNFP-I and 2'-FL (w/w %)	≥ 75.0	89.46	89.89	88.45	80.84	88.71	93.07	88.4
Assay (water-free) – LNFP-I (w/w %)	≥ 50.0	57.70	62.91	70.17	59.92	57.40	63.15	61.9
Assay (water-free) – 2'-FL (w/w %)	≥ 15.0	31.76	26.98	18.29	20.92	31.30	29.92	26.5
L-Fucose (w/w %)	≤ 1.0	< 0.03	< 0.03	< 0.03	0.11	< 0.03	< 0.03	NA
D-Lactose (w/w %)	≤ 10.0	0.44	1.42	0.72	8.56	0.89	0.91	2.2

3-Fucosyllactose (w/w %)	≤ 1.0	0.11	< 0.03	< 0.03	0.03	< 0.03	< 0.03	NA
Difucosyl-D-lactose (w/w %)	≤ 2.0	0.70	0.48	0.28	0.19	0.66	0.68	0.5
Lacto-*N*-tetraose (w/w %)	≤ 5.0	0.65	1.68	3.37	3.21	1.53	1.08	1.9
LNFP-I fructose isomer (w/w %)	≤ 1.5	0.67	0.26	0.22	0.18	0.12	0.16	0.3
2'-Fucosyl-D-lactulose (w/w %)	≤ 1.0	0.60	0.18	0.11	0.18	0.24	0.15	0.2
Assay (water-free) – Specified saccharides a (w/w %)	≥ 90.0	92.65	94.04	93.28	93.82	92.39	96.14	93.7
Sum of other carbohydrates (w/w %)	≤ 6.0	2.64	1.43	1.51	1.53	2.72	1.51	1.9

2'-FL = 2'-fucosyllactose; EU = endotoxin units; LNFP-I = lacto-N-fucopentaose I.

LNFP-I = lacto-N-fucopentaose I; NA = not averaged.

a Sum of specified saccharides includes LNFP-I, 2'-fucosyllactose, lacto-N-tetraose, difucosyl-D-lactose, 3-fucosyllactose, D-lactose, L-fucose, LNFP-I fructose isomer and 2'-fucosyl-D-lactulose.

20. The applicant states that difucosyl-D-lactose, lacto-N-tetraose and 3fucosyllactose are human milk oligosaccharides, with the first two oligosaccharides having approval as novel foods (Annex A: p26 dossier).

21. The applicant states that the low levels of L-fucose, D-lactose, 2’-fucosyl-Dlactulose and LNFP-1 fructose isomer indicate that consumer exposure is expected to be negligible, and not biologically or nutritionally relevant (Annex A: p26 – 27 dossier).

Table 6. Batch Results for water, sulphated ash and pH of the 5% solution of the product for LNFP-I/2'-FL

Parameter	Specification	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6	Average
Water (w/w %)	≤ 8.0	0.78	2.21	2.39	3.96	5.67	2.74	3.0
Sulphated ash (w/w %)	≤ 0.5	0.08	< 0.01	< 0.01	< 0.01	0.10	< 0.01	NA
pH in 5% solution (20°C)	4.0–7.0	4.6	5.9	5.7	5.4	4.4	6.5	NA

NA = not averaged

22. The applicant states that the analysis of the microbiological content of the novel food ingredient confirms the absence of non-pathogenic micro-organisms, selected food borne pathogens including Salmonella, and the production organism (Annex A: p28 dossier).

Table 7. Batch Results for Microbiological Analysis of LNFP-I/2'-FL

Test Parameter (Specification)	Batch 1	Batch 2	Batch 3	Batch 4	Batch 5	Batch 6
Aerobic mesophilic total plate count (≤ 1,000 CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae (≤ 10 CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10
Yeasts (≤ 100 CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10
Moulds (≤ 100 CFU/g)	< 10	< 10	< 10	< 10	< 10	< 10
Salmonella in 25 g (Absence in 25 g)	Absent	Absent	Absent	Absent	Absent	Absent

2’-FL = 2'-fucosyllactose; CFU = colony forming units; LNFP-I = lacto-*N*-fucopentaose I.

23. The methods and validation are provided in Annex B [Annex VI – confidential].

24. The accreditation for the laboratories are found in Annex B [Appendix B2].

Stability

25. The applicant reports the results from a 3-year real-time stability study in bulk at 25oC and 60% relative humidity in two batches of novel food ingredient over a period of three years (Annex A: Table 2.c.3.1-1, p29 dossier). The applicant states that these results confirm the integrity of the product (CoA’s can be found in

Annex B [Annex VII – confidential].

26. The applicant reports the interim results from a 5-year real-time stability study in bulk at 25oC and 60% relative humidity in one batch of novel food ingredient over a period of five years. Results provided for the first nine months only (Annex A: Table 2.c.3.1-2, p30 dossier) show negligible changes in the main parameters.

27. The applicant reports the interim results from a 2-year accelerated stability study in bulk at 40oC and 70% relative humidity in one batch of novel food ingredient over a period of two years. Results provided for the first six months only (Annex A: Table 2.c.3.1-3, p32 dossier) show no appreciable degradation in LNFP-1/2’-FL.

28. The applicant reports the results from a stress and forced stability study under a range of conditions in order to identify potential degradation products (Annex A: p33 – 34 dossier). The data indicates that the bulk product is more stable in dry conditions as the product is hygroscopic under humid conditions. In aqueous conditions, the pH of the solution influences the formation of degradation products (see Table 2.c.3.1-4). The results of these studies are reported in Annex B [Annex VII – confidential].

29. The applicant reports the results from a stability study in powdered infant formula at various temperatures over a period of 12 months (Annex A: Table 2.c.3.2-1, p34 dossier). The results indicate that novel food ingredient shows good stability.

30. The applicant states that based on its structure, the stability of LNFP-I is anticipated to be highly similar to 2'-FL, LNT, and LNnT which have been approved as novel food ingredients in the EU and UK retained under law. Further, 2'-FL has also been tested in the same food systems and evaluated by EFSA as part of its original opinion on 2'-FL (Annex A: p34 dossier).

Specification

31. The specification parameters for the novel food ingredient were assessed using internationally recognised methods or are otherwise determined using internally developed and validated methods – see Table 2.d-1 below (Annex A: p35 dossier).

Table 8. Product Regulatory Specifications for LNFP-I/2'-FL

Parameter	Specification	Method
Appearance	Powder, agglomerates, powder with agglomerates	ISO 6658
Colour	White, white to off-white, off-white	ISO 6658
Identification by Retention Time	RT of main components correspond to RT of standards ± 3%	Glycom method HPLC-13-002
Assay (water-free) – Specified saccharidesa	≥ 90.0 w/w %	Glycom method HPLC-13-001, HPLC-13-002, HPAEC-HMO-017
Assay (water-free) – LNFP-I and 2'-FL	≥75.0 w/w %	Glycom method HPLC-13-002
Assay (water-free) – LNFP-I	≥ 50.0 w/w %	Glycom method HPLC-13-002
Assay (water-free) – 2'-FL	≥ 15.0 w/w %	Glycom method HPLC-13-002
Lacto-*N*-tetraose	≤ 5.0 w/w %	Glycom method HPAEC-HMO-017
3-Fucosyllactose	≤ 1.0 w/w %	Glycom method HPAEC-HMO-017
L-Fucose	≤ 1.0 w/w %	Glycom method HPAEC-HMO-017

D-Lactose	≤ 10.0 w/w %	Glycom method HPAEC-HMO-017
Difucosyl-D-lactose	≤ 2.0 w/w %	Glycom method HPAEC-HMO-017
LNFP-I fructose isomer	≤ 1.5 w/w %	Glycom method HPLC-13-001
2'-Fucosyl-D-lactulose	≤ 1.0 w/w %	Glycom method HPLC-13-001
Sum of other carbohydrates	≤ 6.0 w/w %	Glycom method HPAEC-HMO-017
pH in 5% solution (20°C)	4.0–7.0	Ph. Eur. 2.2.3
Water	≤ 8.0 w/w %	Glycom method KF-001
Ash, sulphated	≤ 0.5 w/w %	Ph. Eur. 2.4.14
Residual protein by Bradford assay	≤ 0.01 w/w %	Glycom method UV-001
Residual endotoxins	≤ 10 EU/mg	Ph. Eur 2.6.14 (LAL kinetic chromogenic assay)
Aerobic mesophilic total plate count	≤ 1,000 CFU/g	ISO 4833-1 or ISO-4833-2

Enterobacteriaceae	≤ 10 CFU/g	ISO 21528-2 or NMKL 144
Salmonella	Absent in 25 g	ISO 6579 or AFNOR BRD 07/11-12/05
Yeasts	≤ 100 CFU/g	ISO 21527-2
Moulds	≤ 100 CFU/g	ISO 21527-2

2'-FL = 2'-fucosyllactose; CFU = colony forming units; ; EU = endotoxin units; HPAEC = high-performance anion exchange chromatography; HPLC =high-performance liquid chromatography; ISO = International Organization for Standardization; LNFP-I = lacto-N-fucopentaose I; Ph. Eur. = European Pharmacopoeia; RT = retention time; KF= Karl Fisher; NMKL = Nordisk Metodikkomite for Levnedsmidler; AFNOR = Association Francaise de Normalisation. a Specified saccharides include LNFP-I, 2'-FL, lacto-N-tetraose, difucosyl-D-lactose, 3-fucosyllactose, D-lactose, L-fucose, LNFP-I fructose isomer and 2'-fucosyl-D-lactulose.

History of Use

32. The applicants indicated that to their knowledge LNFP-I has not been “used” as a food ingredient anywhere in the world to date (Annex A: p36 dossier).

33. The applicant has supplied a summary of the regulatory status of 2'-FL as a food ingredient in different jurisdictions (Annex A: Table 2.e.1-1, p36 – 37 dossier). The applicant provides a summary list of products that use 2'-FL as an ingredient (Annex A: p40 – 45 dossier)

34. The applicant states that the production organism has been used to manufacture a number of EU authorised novel foods and retained in UK law (Annex A: p38 dossier).

35. The applicant provides a review on the human biology of human breast milk with reference to LNFP-I and 2'-FL and the discusses the likely intake levels of these two oligosaccharides based on the consumption of human breast milk (Annex A: p38 – 40 dossier).

Proposed Use and Intake

36. The applicant states that the novel food ingredient is intended to be used by infants, children, and adults, including pregnant and lactating women (Annex A: p46 dossier).

37. The proposed food uses and maximum use levels are below (Annex A: Table 2.f.2-1, p46 – 47 dossier).

Table 9. Proposed Food Uses and Use Levels for LNFP-I from LNFP-I/2'-FL

EU Food Category Number a	Food Category Name	Proposed Maximum Use Level (expressed as LNFP-I)
1	**Dairy Products and Analogues**	
1.1	Unflavoured pasteurised and unflavoured sterilised (including UHT) milk	1.0 g/L
1.2/1.3	Unflavoured fermented milk-based products	1.0 g/L beverages 2.0 g/kg products other than beverages
1.4	Flavoured fermented milk-based products including heat-treated products	1.0 g/L beverages 10.0 g/kg products other than beverages
7	**Bakery wares**	

7.2	Fine bakery wares. Cereal bars only	10.0 g/kg
13	**Foods for Special Groups (FSG)**	
13.1	**Foods for infants and young children**	
13.1.1	Infant formula as defined in Regulation (EU) No 609/2013	1.5 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
13.1.2	Follow-on formula as defined in Regulation (EU) No 609/2013	1.5 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer
13.1.3	Processed cereal-based food and baby food for infants and young children as defined in Regulation (EU) No 609/2013	1.0 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer 8.33 g/kg for products other than beverages
13.1.4	Milk-based drinks and similar products intended for young children	1.2 g/L in the final product ready for use, marketed as such or reconstituted as instructed by the manufacturer 10 g/kg for products other than beverages

13.2	**Foods for special medical purposes as defined in Regulation (EU) No 609/2013**	
13.2	Foods for special medical purposes as defined in Regulation (EU) No 609/2013	On case-by-case basis
13.3	**Total diet replacement for weight control as defined in Regulation (EU) No 609/2013**	
13.3.3	Total diet replacement for weight control as defined in Regulation (EU) No 609/2013	2.0 g/L 20.0 g/kg for products other than beverages
14	**Beverages**	
14.1.4	Flavoured drinks (excluding cola-type drinks)	1.0 g/L

2'-FL = 2'-fucosyllactose; LNFP-I = lacto-N-fucopentaose I; EU = European Union; UHT = ultra-high temperature.

a Numbering in the reference to the Guidance document describing the food categories in Part E of

Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives and the Union List Entry for 2'-FL, See Part E, Annex II

Consolidated version of: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02008R133320200325&from=EN

38. The applicant states that the use level in Table 2.f.2-1 (above) is expressed in terms of LNFP-I added. The corresponding use level of 2’-FL is calculated as 0.428 LNFP-I level (this number is derived from the average content of LNFP-I and 2’-FL

determined by analysis in Table 2.c.2.1-1. So, 2’-FL / LNFP-I = 26.5 / 61.9 = 0.428.

39. The applicant states that the intake of LNFP-I in infants up to the age of 16 weeks at P95 is estimated to be 390 mg/kg body weight/day, equivalent to 2.09 g/day for a 6.7kg infant using EFSA draft formula intake levels (Annex A: p47 dossier).

40. The applicant determined the intake for the novel food ingredient from all conventional foods and beverages (see Annex A: Table 2.f.3.2.2-1, p49 dossier) using the EFSA Comprehensive Database (Annex C: p48 – 49 dossier). Detailed analysis can be found in Annex B [Annex X – confidential].

41. The applicant reports that on a body weight basis, the highest intakes of LNFPI were observed in infants and toddlers, with mean intakes of up to 248 and 123 mg/kg body weight/day, respectively, and high-level intakes at up to 462 and 423 mg/kg body weight/day, respectively. A summary of this analysis can be found in Table 2.f.3.2.3-1 for all population groups (Annex A: p50 dossier).

42. The applicant states that the wide range of intake values at P95 for infants under the age of 11 months represent the variability in the national survey data and the assumption that the proposed foods will be consumed at the maximum proposed intake levels, which is deemed extremely unlikely (Annex A: p51 dossier).

43. The applicant concludes that the intakes of LNFP-I and 2’-FL do not exceed the levels found in human breast milk for which the there is a history of safe use – see Table 2.f.3.3-1 below (Annex A: p50 dossier and Annex B [Annex IX – confidential]).

Table 10. Highest Intakes of LNFP-I and 2'-FL from Breast Milk vs. Highest Level of Exposure from Proposed Uses

Dietary Source	Estimated Daily Intake for LNFP-I (mg/kg bw/day)	Estimated Daily Intake for 2’-FL (mg/kg bw/day)
Mean Intake of Human Milk (800 mla) for 6.7 kg infant		

Highest Mean (based on 4.47 g/L b)	534	863
Highest 95% CL (based on 5.57 g/L b)	665	1,673
Highest Mean Consumption from Proposed Uses b	248	106 d
High Level intake of Breast Milk (1200 mL a) for 6.7 kg infant		
Highest Mean (based on 7.23 g/L b)	801	1,295
Highest 95% CL (based on 14.01 g/L b)	998	2,509
Highest 95th Percentile Consumption from Proposed Food Uses c	462	198 d

2'-FL = 2'-fucosyllactose; bw = body weight; CL = confidence limit; LNFP-I = lacto-N-fucopentaose I.

a EFSA NDA Panel, 2013

b See Section 2.e.3.

c Estimated intake for all infants.

d 2’-FL consumption is calculated from LNFP-I intakes based on the average values in table 2.c.2-1, i.e., 26.5/61.9 = 0.428.

44. The applicant states that the use of the novel food ingredient in food supplements is expected to give estimated intake levels of up to 130 mg/kg bw/day for children 3 – 9 years old, 126 mg/kg bw/day for toddlers and 224 mg/kg bw/day for infants under the age of 11 months (Annex A: p52 dossier).

45. The applicant concludes that consumption of the novel food would lead to significantly lower intake levels for LNFP-I and 2’-FL compared to consumers of breast milk – see Table 2.f.3.4-2 below (Annex A: p53 dossier and Annex B [Annex IX – confidential]).

Table 11. Highest Intakes of LNFP-I and 2'-FL from Breast Milk vs. Highest Level of Exposure from Food Supplements

Dietary Source	Estimated Daily Intake for LNFP-I (mg/kg bw/day)	Estimated Daily Intake for 2’-FL (mg/kg bw/day)
Mean Intake of Human Milk (800 mla) for 6.7 kg infant		
Highest Mean (based on 4.47 g/L b)	534	863
Highest 95% CL (based on 5.57 g/L b)	665	1,673
High Level intake of Breast Milk (1200 mL a) for 6.7 kg infant		
Highest Mean (based on 7.23 g/L b)	801	1,295
Highest 95% CL (based on 14.01 g/L b)	998	2,509
Highest Consumption * from Proposed Infant Food Supplement Use at 1.5 g/day (as LNFP-I) and 6.7 kg Infant a	224	96 d

2'-FL = 2'-fucosyllactose; bw = body weight; CL = confidence limit; LNFP-I = lacto-N-fucopentaose I.

a EFSA NDA Panel, 2013 b See Section 2.e.3.

c 2’-FL consumption is calculated from LNFP-I intakes based on the average values in table 2.c.2-1, i.e., 26.5/61.9 = 0.428.

46. The applicant proposes a series of statements for the labelling of products containing the novel food ingredient to provide assurance that consumer should avoid excessive intake from consuming food products and food supplements together. This is reported to be consistent with the approach mandated for 2’FL (2’-fucosyllactose) on Table 1 of the Union list of novel foods under Commission Regulation (EC) 2017/2340 (Annex A: p53 dossier).

Absorption, Distribution, Metabolism and Excretion (ADME)

47. The applicant states that LNFP-I and 2'-FL in the novel food ingredient are structurally identical to their naturally occurring counterparts in human milk. There is no significant digestion in the upper gastrointestinal tract, limited oral absorption and a small proportion is excreted unchanged. The absorption of the novel food ingredient is not expected to be different from absorption from breast milk and is not expected to pose a safety concern (Annex A: p55 dossier).

Nutritional Information

48. The applicant refers to the EFSA Scientific Opinion on the Essential composition of infant and follow-on formulae which states: “*Human milk oligosaccharides are not considered in the estimation of the energy content of the milk*.”.

49. The applicant proposes labelling the novel food ingredient as *“non-digestible (non-glycaemic) oligosaccharide”*.

Toxicological Information

50. The applicant reports that results from a bacterial reverse mutation assay (OECD TG 471) show that LNFP-I/2’-FL is non-mutagenic at concentrations up to 5,000 µg/plate, in the absence or presence of metabolic activation (Annex A: p58 dossier). The full study report is available in Annex A – References – Gilby, 2020a [unpublished] – confidential.

51. The applicant reports that results from an *in vitro* mammalian cell micronucleus test (OECD TG 487) using LNFP-I/2’-FL is neither clastogenic nor

aneugenic up to a dose of 2000 µg/ml. (Annex A: p58 – 59 dossier). The full study report is available in Annex A – References – Gilby, 2020b [unpublished] – confidential.

52. The applicant reports a sub-chronic (90-day) oral toxicity study in rats utilising the OECD guide line 408 conducted to GLP principles. The LNFP-I/2'-FL was reported to be well tolerated at doses up to 5,000 mg/kg body weight/day (Annex A: p59 – 62 dossier). The full study report is available in Annex A – References – Stannard, 2020 [unpublished] – confidential.

Allergenicity

53. The applicant states that the high purification steps involved in the manufacture are proven to remove protein (*i.e.*, potential allergen) to a level of < 0.0017% w/w (Annex A: p63 dossier).

54. The applicant reports the allergenic potential of introduced proteins as a result of the genetic modification of the micro-organism were assessed using the Allergen Online tool (ver. 20) of the University of Nebraska. No sequence alerts for potential allergenicity were identified – Annex B [Annex II – confidential] for the full amino acid sequences.

Committee Action Required

- The Committee is asked whether the available data provide a satisfactory basis for evaluating the safety of this novel food ingredient.
- If so, the Committee is asked whether it is content to recommend approval of the novel food as an ingredient to be added to the range of foods specified.
- If not, the Committee is asked to indicate what additional data would be required.

Annexes (Confidential)

ACNFP-153-03-Annex A – Dossier and References [Confidential]

ACNFP-153-03-Annex B – Annexes [Confidential]

ACNFP-153-03-Annex C – Request For Information

ACNFP-153-03-Annex D – Applicant's Response to Request For Information